Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [Special Issue V] 2014: 178-184

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Effect of Foliar Spraying of Ascorbic Acid on Chlorophyll a Chlorophyll b, Total Chlorophyll, Carotenoids, Hydrogen Peroxide, Leaf Temperature and Leaf Relative Water Content under Drought Stress in Grapes

Mahtab Zonouri*[1], Taimoor Javadi[2], Nasser Ghaderi[2], Mahmud Khoshesh Saba[2]
[1]Student Master of Horticultural Science, Kurdistan, Iran
[2]Assist. Prof. Department of Horticultural Science, Faculty of Agriculture, University of Kurdistan, Kurdistan, Iran
E-mail: zonouri.mahtab@yahoo.com

ABSTRACT
Grapes as a model for the study of plant ecophysiological responses to drought stress are used. Among the non-enzymatic anti-oxidation ascorbic acid (AsA) is one of the main compounds that has a key role in plant cells. To evaluate foliar spraying of AsA under drought stress, a research during 2012 to 2013 years in the natural environment, and one year after the establishment of the cuttings in clay loam soil was conducted. Cultivars with two-level white seedless and khoshnav, two levels of water stress control (moisture content of 75% field capacity) and drought stress (moisture content of 25% field capacity or irrigation after 7 weeks) with three replications by factorial design in a randomized complete block design were studied.Drought stress statistically significant differences at 1% affected on chlorophyll, carotenoids, leaf temperature and leaf relative water content (RWC) and statistically significant differences at 5% level on hydrogen peroxide. Cultivars in terms of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and hydrogen peroxide, were statistically significant differences at 1%. Responses of cultivars under drought stress (25% fc) and full irrigation (75%fc) were different from each other. Khoshnav in drought stress, higher levels of carotenoids and in full irrigation increased amount of chlorophyll a, chlorophyll b and carotenoids was showed. White seedless in full irrigation, decreased the amount of hydrogen peroxide. While the use of AsA had no effect on chlorophyll, but affected statistically significant at 5% level on interaction of cultivar and AsA, interaction of drought stress and AsA, in addition interaction of AsA and cultivar and drought stress. It is possible that AsA with anti-oxidative properties preventes degradation of chlorophyll and indirectly increased in full irrigation treatments in both cultivars.In drought conditions, RWC of two cultivars showed no significant difference. The use of AsA in drought conditions caused carotenoids further increases in khoshnav. Aaccording to the role of canopy temperature in greater tolerance of low soil moisture, low temperature in canopy khoshnav can indicates a greater potential for maximum photosynthesis and its adaptation to be resistant to dry land conditions. In this aspect has important role during the first years of the establishment of this plant. The results showed that AsA can affect some physiological responses of grapes. Therefore application of AsA significantly protects plants in drought stress.
Keywords: Ascorbic acid, Chlorophyll, Carotenoids

Received 09.06.2014 Revised 30.07.2014 Accepted 25.08. 2014

INTRODUCTION

The world's climate is fast changing; agricultural production is seriously affected by changes in temperature and precipitation patterns [20]. It is anticipated that such changes is affected on available water in arid and semi arid areas of natural habitat. Water protects turgor cells and tissues and enables them to cell division and differentiation. It also has an important role in the transport of crude sap. Drought stress either continuously or temporarily is limited growth and distribution of natural vegetation and more than any other environmental factor has an impact on plants [36]. Water deficit reduces photosynthesis and eventually is caused leaf aging [25]. Plants have developed physiological responses and ecological strategies to overcome water shortage through avoidance or tolerance drought stress [26]. Plant response depends on the nature of water shortages, including short-term changes [45] and accessibility and adaptability to water stress [2]. Nonenzymatic antioxidants such as ascorbic acid and

protective pigments such as carotenoids, are important antioxidants that with involvement of reactive oxygen species prevents the immune system from the negative effects. According to the literature, depending on the species concentrations of the anti-oxidation, during water stress and its rate of increase, decrease or no change has emerged. Part of this contradiction related to a series of events and reactions including the development of drought severity of a widespread drought [23]. It was found in some studies that responses of anti-oxidation and protective pigments of plants against drought stress is quite different and often contradictory [24].Reactive oxygen species (ROS) production sites in plant cells are in chloroplasts, mitochondria and peroxisome [34]. Monodehydroascorbate produced by ascorbate peroxidase directly is an electron acceptor in the photosystem I [45]. Drought stress affected on pigment chloroplast and a decrease in the amount of chlorophyll a and b [27].Anti-oxidation enzymes and AsA in protection of membrane lipids against oxidative damage had become disordered [34]. AsA is effective on regulation of photosynthetic capacity and will affect message processing plant hormones during developmental stages [10]. In addition, affect on activities of feeding cycles in plants and play an important role in the electron transport system and is important as a cofactor in many key enzymes in plants [34]. Stress is depleting supply source of AsA and drought stress induces stomatal closure [24]. This closing of stomata, assimilatory of carbon dioxide limits and NADPH concentration increases due to decreased activity of the K-cycle [8]. Drought stress increases decomposition of Rubisco protein [5]. The decrease in chlorophyll under drought stress mainly due to ROS which can cause damage to the chloroplast [15].Stomatal closure caused by hydrogen peroxide may be reversed by external application of ascorbate, because the ascorbate will neutralize hydrogen peroxide.As a result, plants that have higher levels of AsA responses of them into the abscisic acid (ABA) or hydrogen peroxide may be reduced. Drought stress caused depleting source of AsA and induces stomatal closure [18]. During the summer in the Mediterranean area, plants are exposed to drought stress which there is also associated with high temperatures and intense sunlight (12). Drought stress may increase the formation of ROS that at low concentrations within inter-and intracellular signaling is important. However, when the concentration is high, damages to cellular components (lipids, proteins and nucleic acids). Drought stress is decreased RWC of plant and reduced cell turgure thus growth and development of plant is reduced.Grapes are one of the most important fruits of the world in terms of production and area under cultivation. Among the various cultivars that are grown in Iran, white seedless is the world's best cultivars and its importance in the production of raisins, molasses and fresh table extensive research areas in order to achieve maximum performance and quality of this fruit has prepared extensive research to achieve maximum performance and quality of the fruit.In this context, apart from methods such as breeding programs, the use of chemicals such as AsA as well as a quick, easy and inexpensive method it can be used to increase the quantity and quality of this product. According to Iran is located in arid and semi arid region, drought resistant plants with high performance are essential. To accomplish this, awareness of defensive situation against drought stress is important. Several greenhouse studies to investigate grape biochemical reactions under water stress have done, however, this type of research has performed in natural conditions that confidence in the results and information obtained will be higher. An important feature of these results is tested under natural conditions that increases ensure of present results compared to the greenhouse testing. In this study we have tried to simultaneously drought stress and ascorbic acid on two-years-old vines rooted cuttings established on the natural environment investigate the physiological responses of plants.

MATERIALS AND METHODS

To evaluate the method of foliar spraying of ascorbic acid on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, hydrogen peroxide, leaf temperature and leaf RWC under drought stress in grapes a research during 2012 to 2013 in the research field of Horticultural Science, University of Kurdistan in the natural environment one year after the establishment of the cuttings in clay loam soil was carried out. In this study, cultivars with two-level white seedless and khoshnav, two levels of water stress control (moisture content of 75% field capacity) and drought stress (moisture content of 25% field capacity or irrigation after 7 weeks) with three replications by factorial design in a randomized complete block design were studied. Geographical characteristics of the study area with geographic coordinates 46 degrees 59 minutes east and was 35 degrees 16 minutes north of Sanandaj. Training was conducted in the first year with only two arms per vine and in the second year with two-branch and three buds on each branch was pruned.Leaf temperature during the stress period between the hours of 12:00 to 14:00 by using an infrared thermometer readings and notes. The amount of chlorophyll a, b and carotenoids in leaf samples on the basis of spectroscopic methods and were calculated using a spectrophotometer [30]. The amount of hydrogen peroxide by the reaction of hydrogen peroxide with potassium iodide was

performed. Hydrogen peroxide concentration in milligrams per gram fresh weight of the samples was calculated [1]. Leaf relative water content was calculated by the following equation [16].

$RWC=[(FW-DW)\times(TW-DW)^{-1}]\times 100$

FW, DW and TW respectively fresh weight, dry weight and turgor weight. Information obtained from a factorial experiment in a randomized complete block design with three replications were analyzed using MSTATC and SAS software. Means using Duncan multiple range test with probability of 5% level were compared.

RESULTS AND DISCUSSION

Analysis of variance showed that leaf relative water content under drought stress conditions were significant differences at 1% level and other treatments had no effect on it. Ability to maintain a high RWC at low water potentials may indicate greater strength of the cell wall and its ability to withstand against the loss of water [23]. Significant differences was observed between foliar ascorbic acid 300 mg per liter in white seedless under drought stress and treated with 150 mg l-ascorbic acid under control condition in khoshnav and other treatments were not significantly different (Table 1).The rate of leaf photosynthesis in plants with reduced RWC and leaf water potential decreases [29]. One of the results of AsA deficiency increases abscisic acid, so the application of AsA can prevent increased levels of ABA and prevent the growth inhibitory effects of ABA [39]. The effect of drought stress and benzyl adenine (BA) on Cassia combined AsA and BA on RWC was not effective [49] that these results are consistent with this experiments. Drought stress is decreased RWC of plant and reduced cell turgure thus growth and development of plant is reduced [45]. Several reports on the effect of drought stress on biomass accumulation in plants is provided [12]. Partially increase in dry matter and biomass related to accumulation of water-soluble compounds such as sugars [15]. Water-soluble compounds induce to be enter water into cell or preserves cells from dehydration.

The results showed that leaf temperature was affected by drought stress, cultivar and the interaction of cultivars and drought stress and other treatments had no effect on it. Different genotype structure may have different temperature levels as canopy. Different morphological characteristics such as the color of the plant, the amount of wax, leaf size, possibly through its effect on radiation absorption, thermal conductivity and latent heat flow, or a combination of these factors causes the temperature difference between the two canopy of genotypes [4]. Due to the location of the plant tissues appear to have different temperatures. Leaf photosynthesis is one of the features that will be affected by heat stress [23]. According to the role of canopy temperature in greater tolerance of low soil moisture, low temperature in canopy khoshnav can indicate a greater potential for maximum photosynthesis and its adaptation to be resistant to dry land conditions. In this aspect has important role during the first years of the establishment of this plant (Table 1). When plant is in a proper water relations opens your stomata and evapotranspiration, reduces plant temperature. Increasing temperature leads to inactivation of the enzyme and plant activity is impaired. Because opening of stomatal guard cells is a function of water status of stomatal guard cells can be expected to any change in the amount of water affect the opening and closing of stomata [52]. It seems that temperature increase coincided with drought in both cultivars may be made to is caused by signaling to closing stomata, reducing transpiration and preventing moisture loss (Table 1).During the summer in the Mediterranean area, plants are exposed to drought stress which there are also associated with high temperatures and intense sunlight [34]. Drought stress may increase the formation of ROS that at low concentrations within inter-and intracellular signaling is important however, when the concentration is high, damage to cellular components (lipids, proteins and nucleic acids) [45]. With reduced stomatal conductance, leaf temperature will increase because leaves during transpiration away from their excessive heat [52]. Increase in temperature or loss of humidity often resulting in severe water loss in plants. In addition dry air around the plant to be flow will accelerate the drastic reduction of water in the plant. Such an atmosphere resulting increase in vapour pressure gradient between the leaf and the surrounding air. These factors resulted in an increase in the transpiration rate. In addition, the increase in vapour pressure gradient is accelerated lack of water loss of water in soil [33]. Leaves exposed to drought stress often warmer than the surrounding air temperature because, during the drought stress transpiration that cooling plant is reduced. Leaves of irrigated plants usually cooler than the surrounding temperatures even during the hot hours of the day. Water use per plant in parallel of leaf area expansion increases. Stronger plants than smaller ones, need more water. However, drought is most severe in young vineyards because young plants root systems are less developed and not enough moisture in the soil like the larger plants (9). In research on the reaction temperature in the canopy of grapevines under available and unavailable water conditions results showed that between drought and normal condition there was significant differences in temperature of the canopy but there was no significant difference among canopy temperature of cultivars [43].

The results showed thatchlorophyll a, b and total were affected by drought stress, cultivar and interaction between cultivar and drought stress and other treatments had no effect on them. In the control condition use of AsA did not cause an increase in the amount of chlorophyll a in both cultivars also amount of chlorophyll a in khoshnav was higher than in white seedless. Drought stress reduced the amount of chlorophyll a. In non-stress conditions amount of chlorophyll b in khoshnav was higher than in white seedless. Drought stress reduced the amount of chlorophyll b in khoshnav. The use of ascorbic acid had no significant effect on chlorophyll. The total chlorophyll content decreased under drought stress in this experiment (Table 1).Research shows that plants in response to dehydration closes its stomata closure and in long term leads to degradation of chloroplast. It is followed by a decrease in chlorophyll [6]. It seems that reduction in chlorophyll concentrations due to action of chlorophyllase, peroxidase and compounds phenolics and is the result of chlorophyll degradation [48]. Decrease in chlorophyll concentration in the dehydration condition as ancould be used as a non stomatal limiting factor to be considered. One reason for the decrease in chlorophyll concentration under drought stress conditions increase in chlorophyllase activity that under stress conditions gene expression of this enzyme is induced. Proline levels under drought stress in white seedless increased more than in khoshnav and this is probably has helped further resistance against the destrution of chlorophyll in this cultivar (data was not shown).

It has been reported that proline can prevent destructive activity of chlorophyllase and thereby under drought conditions will prevent the loss of chlorophyll [42]. Based on reports by researchers chlorophyll content in grapes irrigated and dry conditions were alike (7), while other researchers have reported significant reduction in chlorophyll content in grapes under drought stress (11). This contradictioncould based on differences in the degree of stress applied (17), cultivars (53) and the amount of stress is induced (18). Reduction of chlorophyll a and b with increasing drought stress in beans and sugar cane (55) cotton (35) and sunflower (25) was reported that results correspond of the present experiments. The reduction in pigment content due to synthesis of low or rapid degradation occurs is known as one of the symptoms of oxidative stress (51). This phenomenon as light protection mechanism by reducing the absorption of light due to the reduction of pigment content is described (14). The decrease in chlorophyll under drought stress is primarily due to damage to the chloroplast via the effects of ROS (24). Photosynthesis is one of the most important physiological processes in plant that its intensity decreases under drought stress, one reasons of this is that it can damage chlorophyll [21]. Durability photosynthesis and chlorophyll protection in leaves under stress conditions including physiological indicators of stress resistance. During the study effect of drought stress in six Chinese ornamental shrubs total chlorophyll content in two cultivar was more than comparison with other species. Prolonged drought significantly reduced the amount of total chlorophyll (32) that is consistent with this present experiment.The results showed that in terms of carotenoids significant difference in the level of 1% there were among cultivars and AsA also the interaction between and AsA and drought stress, in addition interaction of cultivars and AsA and drought stress were significant at the 5% level and other treatments had no effect on it. In drought conditions levels of carotenoids in khoshnav increased (Table 1).Carotenoids are important anti-oxidation effects on photosynthetic systems [28]. Plants are exposed to drought increases sensitivity to light avoidance and chlorosis in their increases. Oxidative stress affect on the amount of carotenoids in plants. Carotenoids content in apple leaves exposed to stress due to protection of oxidation, light and ROS-induced stress increased. Oxidative stress increases the amount of beta-carotene [2] and this finding was consistent with the present experiments. Carotenoids are able to absorbed high energy of short wavelengths and able to convert singlet oxygen to triplet oxygen and taking the oxygen radicals its anti-oxidation is performed [22]. Carotenoids regulate many physiological processes and plant growth, including the effect on abscisic acid and accumulation of this hormone in plant caused acclimation of plants against environmental stresses [46].

The results showed that hydrogen peroxide affected by drought stress in 5% level and cultivar affected by drought stress in 1% level and other treatments had no effect on it. In present experiments under control condition hydrogen peroxide concentration was reduced in white seedless (Table 1).Hydrogen peroxide as a mediator is involved in ABA transmitting signals that accelerated stomatal closure. ABA increased production of hydrogen peroxide that will accelerate stomatal closure. Stomatal closure caused by hydrogen peroxide may be reversed by external application of ascorbate, because the ascorbate will neutralize hydrogen peroxide [56]. As a result, plants that have higher levels of AsA their reaction may be reduced agains ABA or hydrogen peroxide. Stress is depleting source of AsA and induce stomatal closure [39]. This stomatal closure limits assimilatory of carbon dioxide and increases NADPH concentration through decreased activity of K cycle [8]. Hydrogen peroxide plays a role as an important messenger under drought stress and stomatal closure will accelerate. While strong anti-oxidation of AsA neutralizes hydrogen peroxide. Dehydroascorbatereductase (DHAR), is reduced Dehydroascorbate (oxidized

ascorbate) to ascorbic acid and thus has a role in regulating of reducing ascorbic acid. Guard cells that reduced ascorbic acid levels were higher in them showed less response to signaling of hydrogen peroxide and plants lost more water dehydration under drought stress while stopping DHAR expression will cause increased tolerance to drought [8].

Table 1. Results meanes of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids under drought stress in grapes affected by different foliar ascorbic acid and drought

Cultivar	Treatment	AsA	chlorophyll a (mg/gFw)	chlorophyll b (mg/gFw)	total chlorophyll (mg/gFw)	carotenoids (mg/gFw)
White seedless	Control	AsA0	0.83	0.34	1.16	0.30
		AsA150	1.06	0.44	1.49	0.39
		AsA300	1.03	0.40	1.44	0.36
	Stress	AsA0	0.55	0.28	0.82	0.33
		AsA150	0.48	0.27	0.76	0.16
		AsA300	0.46	0.28	0.74	0.29
Khoshnav	Control	AsA0	1.31	0.62	1.93	0.42
		AsA150	1.59	0.75	2.34	0.49
		AsA300	1.27	0.55	1.82	0.42
	Stress	AsA0	0.60	0.31	0.91	0.25
		AsA150	0.54	0.30	0.83	0.33
		AsA300	0.50	0.27	0.78	0.30

Table 2. Results meanes of hydrogen peroxide, leaf temperature and leaf relative water content under drought stress in grapes affected by different foliar ascorbic acid and drought

Cultivar	Treatment	AsA	hydrogen peroxide (nmol/gFw)	leaf temperature (°C)	leaf relative water content (%)
White seedless	Control	AsA0	1.97	36.3	89.09
		AsA150	1.55	35.7	89.17
		AsA300	1.50	37.0	89.00
	Stress	AsA0	2.02	38.7	85.32
		AsA150	2.12	39.0	87.88
		AsA300	1.91	38.3	84.90
Khoshnav	Control	AsA0	2.04	33.7	86.75
		AsA150	2.06	34.0	89.53
		AsA300	2.30	33.7	88.71
	Stress	AsA0	2.23	37.7	85.96
		AsA150	2.28	39.3	86.09
		AsA300	2.30	39.0	85.63

CONCLUSION

In the present study grapes under combined conditions of drought stress and ascorbic acid were studied. The results showed that drought stress altered physiological traits.Responses cultivars under drought stress (25% fc) and full irrigation (75%fc) were different from each other. Khoshnav in drought stress, higher levels of carotenoids and in full irrigation increased amount of chlorophyll a, chlorophyll b and carotenoids was showed. White seedless in full irrigation, decreased the amount of hydrogen peroxide.Under drought conditions there was no significant difference in leaf relative water so thatthe lowest RWC content of two cultivars was observed about 84.9% in white seedless under drought stress.Aaccording to the role of canopy temperature in greater tolerance of low soil moisture, low temperature in canopy khoshnav can indicate a greater potential for maximum photosynthesis and its adaptation to be resistant to dry land conditions. It is possible that AsA with anti-oxidative properties preventes degradation of chlorophyll and indirectly increased in full irrigation treatments in both cultivars. In this aspect has important role during the first years of the establishment of this plant.The results showed that AsA can affect some physiological responses of grapes. Therefore application of AsA can protects plants in drought stress. Application of AsA through routes such as increased chlorophyll was effective. Due to the key role of AsA as a cofactor in the biosynthesis of various plant growth regulators like GA, ABA, SA and ET, it seems that AsA not only endogenous, but also can affect signaling pathway of this various plant growth regulators and is effective in plant response against various stresses. In addition, the state reduction of ascorbic acid may be involved in signaling of plant hormones. But the value of ascorbic acid in fruit-bearing trees to some environmental stresses during the floral and flowering is not clear.

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CITATION OF THIS ARTICLE

Mahtab Z, Taimoor J, Nasser G, Mahmud K S.Effect of Foliar Spraying of Ascorbic Acid on Chlorophyll a Chlorophyll b Total Chlorophyll, Carotenoids, Hydrogen Peroxide, Leaf Temperature and Leaf Relative Water Content under Drought Stress in Grapes. Bull. Env. Pharmacol. Life Sci., Vol 3 [Spl Issue V] 2014: 178-184